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Determination of Volatile and Semivolatile Contaminants in Meat by Supercritical Fluid Extraction/Gas Chromatography/Mass Spectrometry*

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Abstract: Meat products that were exposed to a warehouse fire were collected and examined to identify contaminants present in the samples. An extraction method using supercritical carbon dioxide at 100 atm and 60°C was developed to analyse and characterise volatile and semi-volatile compounds from the samples. The major volatile compounds were lipid oxidation products, such as hexanal and nonanal. Volatiles concentrations from fire-exposed meat products were compared to control samples to determine compositional differences. Aromatic and polycyclic aromatic hydrocarbons were identified, and naphthalene was measured in suspected fire-damaged meat products. Direct supercritical extraction from the meat samples proved to be a rapid and reproducible method to assess contamination in commercial meat products.

Key words: aldehydes, aromatic hydrocarbons, naphthalene, meat, volatiles.

INTRODUCTION

Chemical contamination in foods and agricultural products occurs at trace levels from diverse sources/actions such as packaging (Gomaa 1993), fire exposure (Howard and Fazio 1980; Johnston *et al* 1994) or irradiation (Nawar 1985). The detection of chemical species uniquely associated with the above processes is often difficult, since many 'marker' analytes either occur naturally in the food matrix or are induced from such treatment as frying, smoking, oxidation or packaging. Chemical analysis methods can supplement sensory analysis to identify products that have been exposed to physical or chemical alteration of foods. The methods can establish a more rational basis for the retention or disposal of contaminated foodstuffs.

* Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable. This article is a US Government work and, as such, is in the public domain in the USA.

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The extraction of volatile and semivolatile components from meats, irrespective of techniques, presents the analyst with a complex array of compounds. Over 274 compounds have been identified from the purge and trap analysis of off-line supercritical fluid extraction (SFE) of beef fat, including acids, alcohols, aldehydes, olefins, enols, ketones, both straight-chain and branched hydrocarbons and lactones (Merkle and Larick 1994). Similar complexity was experienced using direct SFE of raw beef volatiles onto a Tenax trap followed by off-line GC-FID or GC-MS analysis; 86 compounds in the non-condensable fraction of the extract and 59 compounds in the headspace samples of the lipid were found (King M *et al* 1993). Therefore, the problem facing the analyst is to adequately separate the components of the resultant supercritical fluid extract and to ascertain the presence of a compound, which is at a significantly higher concentration in a fire-exposed sample than in the control meat. The determination of unique marker compounds in meat matrices is difficult due to the high lipid, moisture and protein contents of the meat, which makes isolation of individual components difficult. The high lipid content of many meats

can also lead to an array of volatile and semivolatile products due to thermal and/or oxidative induced reactions (Watanabe and Sato 1971; Drumm and Spanier 1991; Spanier and Boylston 1994). Such products, if induced by fire, can be of diagnostic value, provided their level is significantly different from that occurring in products that have not been exposed to fire. Similarly, the analyst may also be able to use the occurrence of pyrolysis products as an indicator of fire exposure, provided the background levels exceed those normally found in the meat matrix or introduced into the meat product by commercial processes, such as smoking. Analytical SFE has been demonstrated to be an efficient and benign technique for the pre-analysis isolation of analytes from food matrices (King 1990; King *et al* 1993; Cygnarowicz-Provost *et al* 1994). SFE with CO₂ has been demonstrated to be an effective technique to concentrate and characterise volatile compounds from a variety of food products (Krukons 1985; Hawthorne *et al* 1988; Snyder and King 1994a,b). SFE also has been used to determine volatile compounds in heated beef (Bailey *et al* 1992; Um *et al* 1992; Merkle and Larick 1994). The integration of SFE with GC-MS permits the rapid detection of volatiles, while extending the range of semi-volatile components that can be analysed (Snyder and King 1994a,b), and offers a technique that can be used for the rapid determination of contaminants in food matrices.

In this research, a method utilising direct SFE of the volatile and semi-volatile contaminants through an injector into a gas chromatograph-mass spectrometer (GC-MS) was developed to identify and quantitate trace marker compounds associated with the exposure of meat to fire and smoke in an underground storage cavern.

MATERIALS AND METHODS

Meat samples

Approximately 300 g of individual meat samples were provided by the Midwest Laboratory of the Food Safety Inspection Service (FSIS) (St Louis, MO, USA). These samples were obtained initially from a food storage cavern which was partially consumed by fire at the Americold Corporation, (Kansas City, KS, USA). These suspect samples involved the potential contamination of 300 million pounds of food including 50 million pounds of meat. The samples were stored between -10 and -20°C before use. Seven meat products that had been exposed to fire and smoke damage and their respective control samples were used in this study. The control samples were obtained from products of similar composition but produced at different times and locations. The products included roast beef,

boneless beef trimmings, corned beef, ham, smoked chicken, boneless turkey breast and roast turkey breast.

Standards

Aromatic hydrocarbons including polycyclic hydrocarbons (PAH) were purchased from Aldrich Chemical Co (Milwaukee, WI, USA). Deuterated compounds were purchased from Supelco Inc (Bellefonte, PA, USA). Aldehyde compounds were obtained from Bedoukian Chemicals (Danbury, CT, USA).

SFE concentration of the volatile compounds

Samples were extracted and analysed using supercritical carbon dioxide (SC-CO₂) followed by GC-MS. The meat sample (0.5 g) was weighed into a 5 ml extraction cell and held in place by glass wool plugs on both ends of the cell. The cell was inserted into a Suprex PrepMaster SFE unit (Suprex Corp), Pittsburgh, PA, USA) for SFE. A heated transfer line was constructed connecting the cell and a 50 µm frit restrictor (Dionex Corp, Salt Lake City, UT, USA). The restrictor was inserted into the injector to introduce the extracted compounds into a Varian 3400 GC (Varian, Sunnyvale, CA, USA). The extractor cell and transfer line were maintained at 60°C and 100 atm for the extractions. Extraction conditions were optimised to limit the amount of coextracted lipids (Snyder 1995). The flow rate of the expanded CO₂ during dynamic SFE into the GC was measured at 40 ml min⁻¹. After static SFE for 5 min, the volatiles were transferred onto a capillary column (held at -50°C) over a 0.5 min period using dynamic SFE. The DB-1701 capillary column (0.25 mm, 30 m, 0.1 µm film thickness) (J&W Scientific, Folsom, CA, USA) was then held cryogenically with liquid nitrogen at -50°C for 1 min. The column temperature was then programmed to 250°C at 5°C min⁻¹. Helium was the carrier gas with a flow rate of 0.5 ml min⁻¹. The volatile and semi-volatile components were analysed and identified using a Finnigan Incos 50B Mass Spectrometer (Finnigan Corp, San Jose, CA, USA) operating in the EI mode. An ionisation voltage of 70 eV over the mass range of 20-450 amu was used to fragment the components.

Quantitation

Naphthalene calibrations were done from 1 to 500 ppb, using selective ion monitoring at 128 amu. Naphthalene concentrations in the meat products were determined

from the calibration curves over the above concentration ranges, using hexadecane as an internal standard. Another method using naphthalene-d8 was also employed for determining the amount of naphthalene in the meat samples. Recovery data for the SFE from meat samples, utilised a 50 ppb deuterated naphthalene as an internal standard in the meat samples. Ratios at 128 amu (for naphthalene) and 136 amu (for naphthalene-d8) were used to determine the concentration of naphthalene in each meat product.

Aromatic hydrocarbon and aldehyde concentrations were determined from calibration curves of standard compounds described previously by Snyder and King (1994a).

RESULTS AND DISCUSSION

The total ion chromatogram (Fig 1) from GC-MS analysis of a smoked chicken product demonstrates the wide range of compounds present in the smoked chicken product that had been exposed to fire. Lipid oxidation products, such as hexanal, nonanal, octanal, pentylfuran and decadienal isomers, are found to be major components in the GC-MS profiles. Off-line SFE experiments on vegetable oils (Snyder and King 1994a,b) and additional similar off-line SFE-GC-MS experiments on several meat samples, have consistently shown that SFE is more effective than purge and trap methodology in extracting compounds with carbon numbers above 6. Contaminants associated with fire or smoke exposure in this GC run included the aromatic hydrocarbons: toluene, ethylbenzene, xylene, naphthalene and phenanthrene. Dimethylpyrazine was also apparent and has been found in foods exposed to

heat (Watanabe and Sato 1971). The occurrence of this nitrogen-containing moiety is probably due to the heating of protein matter in the meat matrix. The presence of di-*n*-butyl phthalate was probably due to the surrounding package material. Nonanoic and hexadecanoic acids, shown in the chromatogram, were the major fatty acids extracted from these meat samples.

Major lipid oxidation products increased in most of the meat samples that were exposed to fire, as shown by the hexanal concentration (Fig 2) and the nonanal concentration (Fig 3). In this case, hexanal and nonanal had the highest concentrations of all the volatile and semi-volatile compounds found in the meat samples. Hexanal and nonanal increased in five of the seven meat products exposed to the fire; however, both aldehydes were present in lower concentrations in the fire-exposed boneless beef and turkey breast than in the control samples.

Naphthalene was monitored in the supercritical fluid extracted meats to assess the effectiveness of the SFE-GC-MS technique for the detection of this fire-associated marker compound. Naphthalene is a particularly appropriate marker compound, since it has a high solubility in SC-CO₂ and has been studied extensively (Krichevskii *et al* 1971; Tan and Weng 1987; Mitra *et al* 1988). As shown in Table 1, there is a definite difference between the naphthalene content of the control samples and those exposed to fire, when the SFE-GC-MS method is employed for analysis. This supports the use of naphthalene as a marker component for fire exposure.

The results obtained by using a deuterated naphthalene internal standard (Table 2) show a similar trend to those given in Table 1, namely a significant increase in the naphthalene content for samples that have been exposed to fire. There are differences in the results for

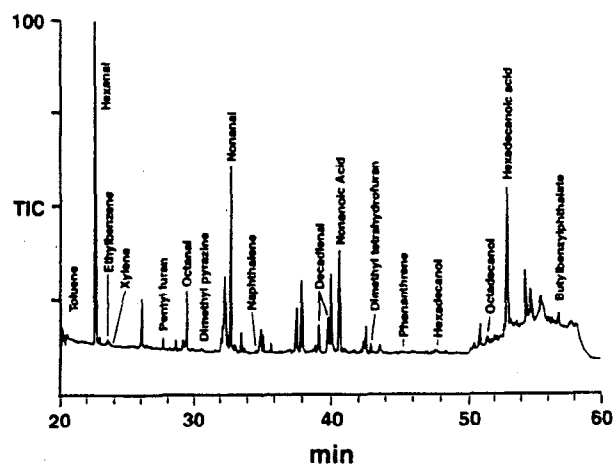


Fig 1. Total ion chromatogram from SFE/GC/MS of the smoked chicken product that was exposed to fire.

TABLE 1

Concentration^a of naphthalene in meat samples by SFE/GC/MS

Meat Type	Control		Fire-exposed	
	ppb	RSD ^b	ppb	RSD ^b
Beef roast	0	—	13.0	8.1
Boneless beef	LOQ ^c	—	LOQ	—
Corn beef	LOQ	—	7.6	7.4
Ham	5.4	10.6	21.3	5.6
Smoked chicken	10.3	6.6	39.2	5.4
Turkey breast	LOQ	—	4.7	7.4
Boneless turkey	0	—	5.8	5.0

^a Concentration determined by response curves.

^b RSD, Relative standard deviation determined from three extractions.

^c LOQ, limit of quantitation is 1 ppb.

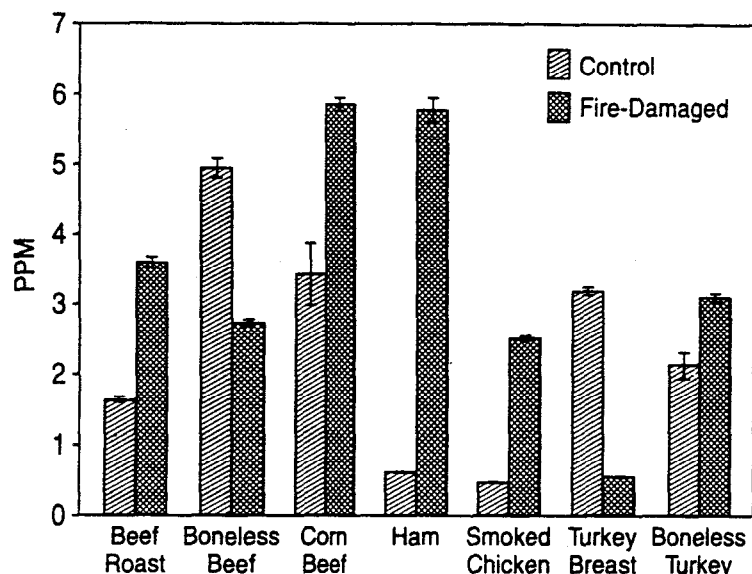


Fig 2. Concentration of hexanal from the control and fire-exposed meat samples.

the two methods used for quantitation, as shown in Tables 1 and 2. In Table 1, there are three samples (control and fire-damaged boneless beef and corned beef control), which have only a trace of naphthalene according to the external standard method. However, naphthalene could be determined, more accurately at lower levels, using the deuterated, internal standard naphthalene method (Table 2). Comparison of the mean naphthalene concentration of each meat type from Table 2, with that of the corresponding meat type in Table 1 by a paired *t*-test demonstrated that the difference in methods was not significant at $P < 0.05$. The quantitation method using deuterated naphthalene as

an internal standard (Table 2) was a more precise and sensitive method than the external standard method for determining naphthalene in the fire-exposed meat products. Other aromatic hydrocarbons that were also identified in the fire-exposed meats were toluene, ethylbenzene and xylene (Table 3). Various alkylated benzenes have also been found in roast beef by Min *et al* (1977). Their quantitative study confirmed the presence of toluene, ethylbenzene and xylenes in the roast beef extracts, and they speculated that the occurrence of such compounds could be due to the thermal degradation of amino acids or the breakdown of co-extracted lipid species, such as *trans*-2-*trans*-4-decadienal.

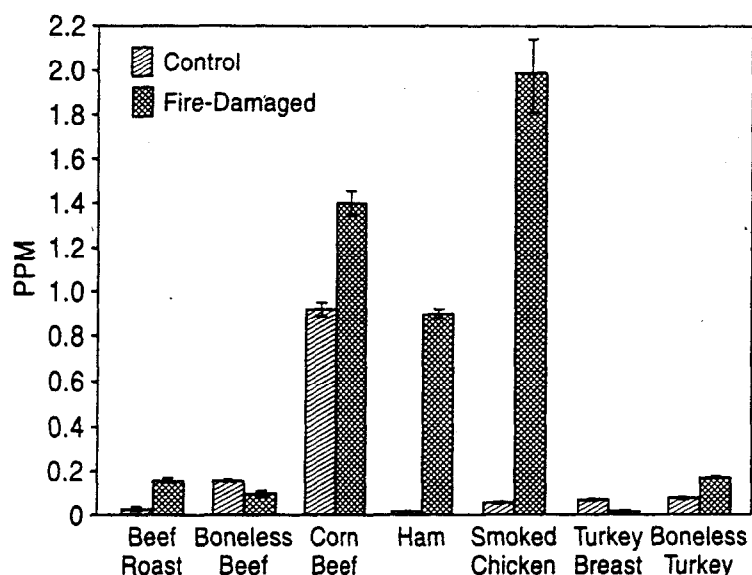


Fig 3. Concentration of nonanal from the control and fire-exposed meat samples.

TABLE 2

Concentration^a of naphthalene in meat samples by SFE/GC/MS

Meat Type	Control		Fire-exposed	
	ppb	RSD ^b	ppb	RSD ^b
Beef roast	0	—	10.7	8.5
Boneless beef	LOQ ^c	—	3.5	4.1
Corn beef	1.7	14.2	14.6	3.1
Ham	2.5	12.8	21.3	4.8
Smoked chicken	11.7	5.3	50.8	0.6
Turkey breast	LOQ	—	4.3	4.0
Boneless turkey	0	—	6.2	0.8

^a Concentration determined using naphthalene-d8 as internal standard.^b RSD, relative standard deviation was determined from 3 extractions.^c LOQ, Limit of quantitative is 1 ppb.

Although these compounds were found in concentrations less than 5 ppb in the smoked chicken control and a trace amount of toluene was found in the ham control, only the five fire-damaged meat samples listed in Table 3 had detectable amounts of these compounds. The above three aromatic compounds were also several times more concentrated in the smoked chicken sample that was exposed to the fire. Therefore, these marker compounds also appear to offer promise as diagnostic aids in detecting fire exposure in meats.

SUMMARY AND CONCLUSION

A SFE-GC-MS method was developed for the determination of chemical contaminants using 'marker' compounds, specifically naphthalene, down to ppb level in

TABLE 3

Aromatic compounds in fire-exposed meats by SFE/GC/MS

Meat type	Concentration (ppb)		
	Toluene	Ethylbenzene	Xylene
Boneless beef	20.3 (0.6) ^a	11.1 (0.9)	—
Corn beef	51.8 (2.2)	31.0 (1.4)	19.4 (0.7)
Ham	80.2 (0.3)	41.7 (0.7)	15.0 (3.1)
Smoked chicken	329.3 (0.1)	250.5 (0.1)	163.2 (0.8)
Turkey breast	36.1 (2.8)	11.0 (5.4)	14.2 (3.5)

^a RSD, Relative standard deviation determined from 3 extractions given in parentheses.

the presence of other volatile coextractives in meats exposed to fire or smoke.

Exposure to fire of suspect samples taken from a food storage cavern fire was detected by monitoring the increases in both volatile carbonyl components and aromatic hydrocarbons in the supercritical extract using the SFE-GC-MS technique. The method appears to be applicable to solving many contamination problems which occur in various food matrices, and can be used as a confirmatory and supplementary method which is superior to simple sensory analysis.

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